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Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands

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1. The effect of Li^+ on the agonist-dependent metabolism of [^{3}H]inositol has been studied in rat brain, rat parotid and the insect salivary gland. 2. When brain or parotid slices were incubated in the presence of [^{3}H]inositol, Li^+ was found to amplify the ability of agonists such as carbachol, phenylephrine, histamine, 5-hydroxytryptamine and Substance P to elevate the amount of label appearing in the inositol phosphates. 3. A different approach was used with the insect salivary gland, which was prelabelled with [^{3}H]inositol. After washing out the label, the subsequent release of [^{3}H]inositol induced by 5-hydroxytryptamine was greatly decreased by Li^+. During Li^+ treatment there was a large accumulation of [^{3}H]inositol 1-phosphate. 4. This ability of Li^+ to greatly amplify the agonist-dependent accumulation of *myo*-inositol 1-phosphate offers a novel technique for identifying those receptors that function by hydrolysing phosphatidylinositol. 5. The therapeutic action of Li^+ may be explained by this inhibition of *myo*-inositol 1-phosphatase, which lowers the level of *myo*-inositol and could lead to a decrease in the concentration of phosphatidylinositol, especially in those neurons that are being stimulated excessively. This alteration in phosphatidylinositol metabolism may serve to reset the sensitivity of those multifunctional receptors that generate second messengers such as Ca^{2+}, cyclic GMP and the prostaglandins.

Lithium is used extensively in the control of manic-depressive illness despite the fact that its mode of action is still unknown. Many of the drugs that induce or reverse this illness interfere with the physiology of biogenic amines, suggesting that the changes in mood associated with depression or mania may be associated with alterations in amine neurotransmission (Schou, 1976). A major challenge in this field is to identify the biochemical target for the action of lithium.

A potential breakthrough was made by Allison & Stewart (1971) when they discovered that Li^+ decreased the concentration of *myo*-inositol in the cerebral cortex of rats. Further studies revealed that this Li^+-induced decrease in *myo*-inositol was associated with very large elevations in the concentration of *myo*-inositol 1-phosphate resulting from an inhibition of the enzyme *myo*-inositol 1-phosphatase (Allison & Blisner, 1976; Allison *et al.*, 1976; Allison, 1978; Hallcher & Sherman, 1980; Sherman *et al.*, 1981). On the basis of these observations it has been suggested that Li^+ may exert its therapeutic action by interfering with the metabolism of the phosphoinositides, which are known to play an important role in synaptic function (Sherman *et al.*, 1981).

The hydrolysis of phosphatidylinositol is an integral part of those receptors (e.g. muscarinic, H_1-histaminergic, α_1-adrenergic and V_1-vasopressin receptors) that function through Ca^{2+} (Michell, 1975, 1979; Berridge, 1980, 1981; Michell & Kirk, 1981; Putney, 1981). These receptors are multifunctional in that they may also be responsible for generating cyclic GMP and for releasing arachidonic acid (Berridge, 1981). Li^+ may thus exert its therapeutic action by altering the activity of this important class of receptors. Since these receptors have been examined more extensively in peripheral tissues, we decided to see whether Li^+ exerted a similar effect on inositol metabolism in the brain and in both insect and mammalian salivary glands. The results reported in the present paper not only provide new insights into the therapeutic action of Li^+ but they also suggest that Li^+ can be used to provide a sensitive method for identifying the important class

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of receptors that function through Ca^{2+} rather than through cyclic AMP.

Methods

Incubation conditions

Salivary glands were isolated from adult female blowflies (*Calliphora erythrocephala*). The technique used to measure the release of [^{3}H]inositol from prelabelled glands was identical with that described by Fain & Berridge (1979).

The dissection and preparation of rat parotid-gland fragments have been described previously (Hanley *et al.*, 1980). For preparation of brain slices, male Sprague–Dawley rats were decapitated and the cerebral cortex hand-dissected on ice. Cross-chopped slices (width 350μM) were cut with a McIlwain tissue chopper. The slices were washed thoroughly in warm Krebs Ringer bicarbonate containing 10mM-glucose (KRB) and then incubated for 30min in this buffer with gentle shaking in a 37°C water bath. They were then washed again in KRB buffer and finally allowed to settle under gravity.

Labelling and extraction of water-soluble inositol phosphates

Insect salivary gland. 120 salivary glands were incubated in 1ml of control medium containing 5μM-*myo*-[2-^{3}H]inositol for 2h, thoroughly washed and separated into groups of six in small glass vials. Control groups were incubated for various times in 200μl of control medium, whereas the experimental groups were treated with 200μl of control medium containing 0.5μM-5-hydroxytryptamine, 5mM-Li^+ or a combination of the two. The incubations were terminated by the addition of 1ml of chloroform/methanol (1:2, v/v). Chloroform was added to separate the phases and the upper phase was removed and dried.

Rat brain and parotid gland. Experiments with brain slices and parotid fragments were essentially similar. Portions (50μl) of the gently packed tissue preparations were pipetted into Beckman Biovials containing 0.32–0.64μM-*myo*-[2-^{3}H]inositol in 250μl of incubation medium. When present, LiCl replaced NaCl in the normal incubation medium to give the appropriate Li^+ concentration. Drug additions were made from stock solutions in KRB buffer. The vials were gassed (O_2/CO_2, 19:1) and capped and then incubated at 37°C in a gently shaking water bath. The incubations were stopped by addition of 0.94ml of chloroform/methanol (1:2, v/v). Chloroform (0.31ml) and 0.31ml of water were then added to separate the phases. A portion (0.75ml) of the upper phase was removed for analysis of water-soluble [^{3}H]inositol-labelled compounds.

Simplified assay of total labelled inositol phosphate fraction. This method was used to derive the results on rat brain and parotid glands. Samples of the aqueous phase obtained as described above were diluted to 3ml with water. A 50% (w/v) slurry of Dowex-1 (0.5ml; 100–200 mesh; X8) in the formate form was then added to bind inositol phosphates. The resin was allowed to settle and the supernatant was discarded. After washing the resin four times each with 2.5ml of 5mM-*myo*-inositol, inositol phosphates were eluted with 0.5ml of 1M-ammonium formate/0.1M-formic acid. Of this eluate, 0.4ml was then removed and radioactivity was determined by scintillation counting.

Chromatographic separation of water-soluble inositol phosphates

Water-soluble extracts prepared as described above were applied to Whatman no. 1 chromatographic paper, which was subjected to high-voltage ionophoresis in a 0.1M-ammonium carbamate/ammonium acetate buffer, pH9.0 (Clarke & Dawson, 1981). After the paper had been dried at 70°C each track was divided into 1cm segments, which were treated overnight with 1ml of water. After separation into two equal parts, one part was used to identify the position of label in the inositol phosphates, whereas the remaining portions were digested in $HClO_4$ and their phosphorus contents were determined by the procedure of Bartlett (1959) to identify the position of standard *myo*-inositol 1-phosphate.

Water-soluble extracts were also applied to columns containing 1ml of Dowex-1 (X8; Sigma Chemical Co., London S.W.6, U.K.) in the formate form. The phosphate esters were then eluted by the stepwise addition of solutions containing increasing concentrations of formate. Glycerophosphoinositol and inositol 1:2-cyclic phosphate were eluted with 5mM-sodium tetraborate plus 60mM-sodium formate, whereas *myo*-inositol 1-phosphate was eluted with 5mM-sodium tetraborate plus 150mM sodium formate (Richards *et al.*, 1979). The penultimate solution contained 0.1M-formic acid plus 0.3M-ammonium formate, followed by 0.1M-formic acid plus 0.75M-ammonium formate, each of which removes more polar inositol phosphates (Downes & Michell, 1981). The 1ml fractions eluted from the columns were counted for radioactivity after addition of 10ml of Biofluor.

Results

The inhibitory effect of Li^+ on *myo*-inositol 1-phosphatase will lead to the accumulation of *myo*-inositol 1-phosphate with a corresponding decline in the concentration of *myo*-inositol. Such changes in the level of these water-soluble components have been studied on a number of tissues

using different techniques. Preliminary experiments were designed to detect the accumulation of inositol phosphates by measuring these labelled compounds in the water-soluble fraction obtained from brain and parotid during stimulation with various agonists. The ability of Li^+ to decrease the release of [^{3}H]inositol from the insect salivary gland was used to illustrate how this ion can lower the intracellular concentration of *myo*-inositol. These indirect studies were supported by direct measurements of the effect of Li^+ on the intracellular concentration of *myo*-inositol 1-phosphate

The effect of Li^+ on the labelling of inositol phosphates in cerebral cortex and parotid gland

When slices of cerebral cortex were incubated in [^{3}H]inositol there was a gradual linear accumulation of label in the inositol phosphates (Fig. 1). The addition of either carbachol or Li^+ caused a slight increase in the labelling but when added together there was a very large effect (Fig. 1). There appeared to be a short latency, but after 10min the radioactivity in the inositol phosphates increased linearly over the 60min incubation period. This ability of carbachol to enhance the labelling of inositol phosphates was inhibited by atropine (Table 1). A similar enhancement of labelling was observed with histamine, 5-hydroxytryptamine and phenylephrine but not with isoprenaline.

Similar experiments were carried out on the rat parotid gland, which possesses α_1- and β-adrenoreceptors, muscarinic acetylcholine receptors and receptors for the undecapeptide, Substance P. Only the α_1-, muscarinic and Substance P receptors are coupled to inositol phospholipid breakdown. When added by themselves carbachol and phenylephrine had the largest effect, whereas that of Substance P was very small and isoprenaline was without effect (Table 2). Li^+ by itself was slightly stimulatory, but, when added in combination with some of the agonists, it greatly enhanced the incorporation of label into the inositol phosphates. The greatest potentiation was seen with carbachol, whereas the effects with phenylephrine and Substance P were somewhat smaller.

The effect of Li^+ on inositol release from Calliphora salivary glands

When isolated salivary glands are incubated with [^{3}H]inositol for 2h and then washed extensively,

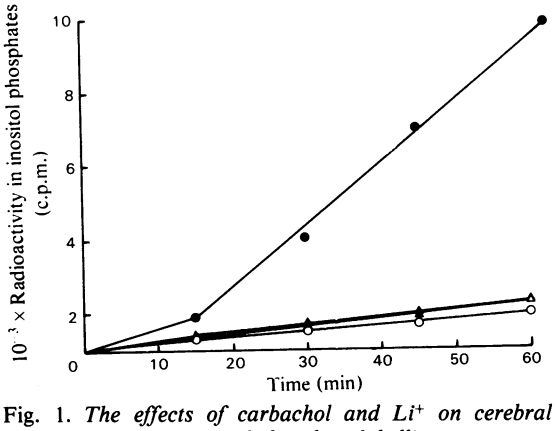

Fig. 1. *The effects of carbachol and Li^+ on cerebral cortex inositol phosphate labelling*
Brain slices were incubated for the times indicated with 0.64μM-*myo*-[^{3}H]inositol and no further additions (○); plus 0.1mM-carbachol (△); plus 10mM-Li^+ (▲); and plus 0.1mM-carbachol and 10mM-Li^+ (●). The incubations were started by addition of the tissue and terminated by adding chloroform/methanol (1:2, v/v). Radioactivity in the total inositol phosphate fraction was determined. Each point is the mean of duplicate incubations in a single representative experiment.

Table 1. *Neurotransmitters that stimulate inositol phosphate accumulation in Li^+-treated rat cerebral cortex slices*
Brain slices were incubated for 30min as described for Fig. 1 with 10mM-Li^+ and the drugs indicated. Radioactivity in the total inositol phosphate fraction was assayed. The results are means ± S.E.M. for three separate experiments.

Drug addition	Stimulation of inositol phosphate labelling (% of control)
None	100
Carbachol (0.1mM)	420 ± 70
Atropine (10μM)	100 ± 5
Carbachol (0.1mM) + atropine (10μM)	110 ± 5
Histamine (0.1mM)	160 ± 4
5-Hydroxytryptamine (0.1mM)	180 ± 20
Phenylephrine (0.1mM)	200 ± 7
Isoprenaline (0.1mM)	84 ± 10

Table 2. *The influence of Li^+ and receptor activation on inositol phosphate labelling in rat parotid glands*
Parotid fragments were incubated with 0.32μM-*myo*-[2-^{3}H]inositol and the drugs indicated for 30min. The results, expressed as radioactivity in the total inositol phosphate fraction, are means ± S.E.M. for triplicate determinations in a single experiment. Similar results were found in two other experiments.

	Radioactivity in inositol phosphates (c.p.m.)	
Drug addition	Control	Plus Li^+ (10mM)
None	120 ± 10	150 ± 2
Carbachol (0.1mM)	354 ± 10	2520 ± 260
Phenylephrine (0.1mM)	210 ± 40	580 ± 40
Substance P (2μM)	160 ± 30	390 ± 15
Isoprenaline (0.1mM)	120 ± 10	220 ± 60

more than 95% of the label retained by the tissue is in the form of [^{3}H]phosphatidylinositol (Fain & Berridge, 1979). The release of [^{3}H]inositol from such prelabelled glands is very small in control medium but is greatly increased by the addition of 0.5 μM-5-hydroxytryptamine (Fig. 2). The enhanced release of [^{3}H]inositol observed during the action of 5-hydroxytryptamine is markedly decreased by treatment with 5 mM-Li^+ (Fig. 2). This inhibitory effect on inositol release is consistent with the hypothesis that Li^+ acts by blocking the enzyme that converts *myo*-inositol 1-phosphate into inositol. Another consequence of inhibiting this enzyme is that *myo*-inositol 1-phosphate will accumulate within the cell. When Li^+ is withdrawn, the accumulated *myo*-inositol 1-phosphate will be converted into *myo*-inositol, which probably accounts for the pronounced compensatory release of [^{3}H]inositol during recovery from the Li^+ treatment in Fig. 2.

Effect of varying Li^+ concentration

The serum and brain levels over which lithium exerts its therapeutic action in controlling manic-depressive illness are rather narrow (0.8–1.2 mM) (Ehrlich & Diamond, 1980). It was of considerable interest, therefore, to determine the dose–response relationships for Li^+ for the receptor-mediated events described above. The effect of various Li^+ concentrations on the ability of carbachol to stimulate the accumulation of label in cerebral cortex and parotid was remarkably similar to its effect on the release of inositol from the insect salivary gland stimulated by 5-hydroxytryptamine (Fig. 3). Li^+ was half-maximally effective at about 1 mM, which lies within the normal therapeutic range (0.8–1.2 mM) for serum concentrations of this drug. Since the intracellular level of Li^+ in most cells is usually somewhat higher than those in serum (Ehrlich & Diamond, 1980), the dose–response curves in Fig. 3 are in keeping with the previous observation that about 0.7 mM-Li^+ half-maximally inhibited *myo*-inositol 1-phosphatase *in vitro* (Sherman *et al.*, 1981).

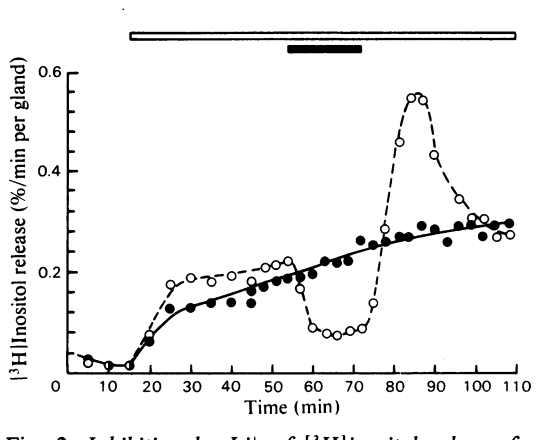

Fig. 2. *Inhibition by Li^+ of [^{3}H]inositol release from blowfly salivary glands*
Groups of three salivary glands were incubated with 5 μM-*myo*-[2-^{3}H]inositol for 2h. The glands were washed and the release of label was measured during stimulation with 0.5 μM-5-hydroxytryptamine (open bar). One group of glands (○) was treated with 5 mM-Li^+ for 18 min (filled bar). The label released from the glands at set intervals was expressed as a percentage of the total label present in the glands during each efflux period.

The accumulation of myo-inositol 1-phosphate during the action of Li^+

If Li^+ acts by inhibiting the *myo*-inositol 1-phosphatase it should lead to the accumulation of *myo*-inositol 1-phosphate. The latter has been identified by using a combination of high-voltage ionophoresis and anion-exchange chromatography. The water-soluble fraction obtained after stimulating the insect salivary gland for 5 min with 5-hydroxytryptamine (0.5 μM) in the presence of 5 mM-Li^+ was mixed with a sample of *myo*-inositol 1-phosphate and subjected to high-voltage iono-

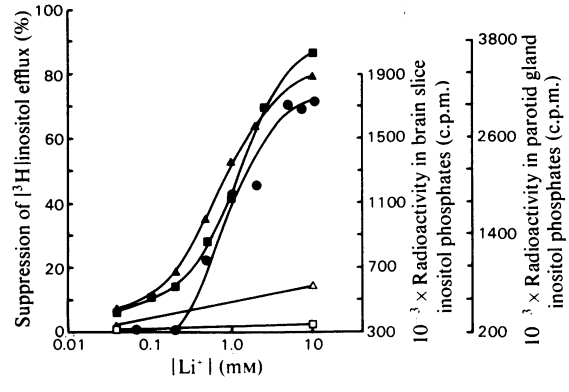

Fig. 3. *Dose–response curves for Li^+-induced changes in inositol metabolism in brain slices and salivary glands*
Brain slices (▲ and △) and parotid gland fragments (■ and □) were incubated for 30 min as described in the legends to Fig. 1 and Table 2 respectively. The Li^+ concentration was varied either with (filled symbols) or without (open symbols) 0.1 mM-carbachol. Radioactivity in the total inositol phosphate fraction was assayed. Groups of three blowfly salivary glands were prelabelled as described in the legend to Fig. 2. They were stimulated with 0.5 μM-5-hydroxytryptamine for 40 min to establish a constant rate of [^{3}H]inositol efflux. The glands were then treated with different concentrations of Li^+ and the extent to which the efflux of [^{3}H]inositol was decreased was expressed as a percentage of the control efflux in the absence of Li^+ (●). Very similar dose–response curves were obtained in several separate experiments.

phoresis. The sample of *myo*-inositol 1-phosphate was found to co-migrate with a radioactive spot that ran at a position of 0.76 relative to P_i, which is very close to the value of 0.75 reported by Clarke & Dawson (1981). The radioactivity that was located at this position was extracted from the paper and was found to be eluted from the anion-exchange columns at the same ionic strength as that used by Richards *et al.* (1979). These anion-exchange columns were then used to measure the accumulation of *myo*-inositol 1-phosphate in the insect salivary gland during stimulation with 5-hydroxytryptamine either in the presence or absence of Li^+.

The ability of these anion-exchange columns to separate *myo*-inositol 1-phosphate from the remaining inositol phosphates is shown in Fig. 4. As expected, Li^+ suppressed the formation of free inositol that appears in the first peak. It had no effect on the level of the second peak, which is likely to be glycerophosphoinositol. The most pronounced effect of Li^+ was found in the third region, which corresponds to *myo*-inositol 1-phosphate as described above. Experiments with the rat parotid gland and brain slice preparations (results not shown) gave remarkably similar results to those described for the insect salivary gland. Concomitant with carbachol stimulation, Li^+ greatly enhanced the size of the *myo*-inositol 1-phosphate peak eluted from Dowex-1 columns. The remaining two peaks have not been identified. Li^+ was found to slightly enhance the labelling of peak IV, but had no effect on peak V.

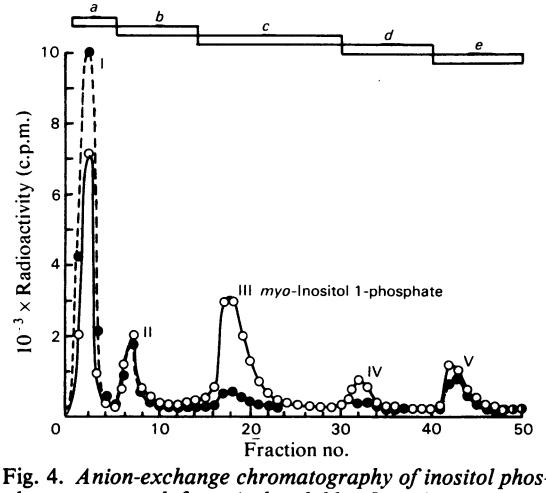

Fig. 4. *Anion-exchange chromatography of inositol phosphates extracted from isolated blowfly salivary glands during stimulation with 5-hydroxytryptamine and Li^+*

Groups of six glands were incubated in 5 μM-*myo*-[2-^{3}H]inositol for 2h. The glands were washed and then stimulated for 5min with 200μl of 0.5 μM-5-hydroxytryptamine either in the absence (●) or the presence (○) of 5mM-Li^+. The incubations were stopped by adding 1ml of chloroform/methanol (1:2, v/v). The water-soluble components were applied to Dowex-1 anion-exchange resin and eluted with: (*a*) water; (*b*) 5mM-sodium tetraborate/60mM-sodium formate; (*c*) 5mM-sodium tetraborate/150mM-sodium formate; (*d*) 0.1M-formic acid/0.3M-ammonium formate; (*e*) 0.1M-formic acid/0.75M-ammonium formate.

The time course for the changes in accumulation of [^{3}H]inositol and [^{3}H]inositol 1-phosphate is shown in Fig. 5. In the absence of 5-hydroxytryptamine, Li^+ caused no change in the accumulation of [^{3}H]inositol (Fig. 5*a*) and a very small accumulation of [^{3}H]inositol 1-phosphate (Fig. 5*b*, inset). In the absence of Li^+, 5-hydroxytryptamine stimulated a small increase in the concentration of [^{3}H]inositol 1-phosphate (Fig. 5*b*) and a very large accumulation of [^{3}H]inositol (Fig. 5*a*), most of which was probably released to the bathing medium. When glands were treated with a combination of Li^+ and 5-hydroxytryptamine, the accumulation of [^{3}H]inositol was markedly suppressed (Fig. 5*a*), whereas the concentration of [^{3}H]inositol 1-phosphate rose to very high levels (Fig. 5*b*).

The dose–response curve for the effect of 5-hydroxytryptamine on the accumulation of [^{3}H]-*myo*-inositol 1-phosphate in the insect salivary gland provides further evidence for the ability of Li^+ to amplify agonist-dependent responses (Fig. 6). In the absence of Li^+, the accumulation of this phosphate is small and barely detectable below a dose of 10nM, even though this level of 5-hydroxytryptamine can induce a maximal secretory response (Berridge, 1970). In the presence of Li^+, however, the accumulation of this phosphate is very much larger, especially at the higher concentrations of 5-hydroxytryptamine (Fig. 6). The divergence of the two curves as the 5-hydroxytryptamine concentration increases indicates that Li^+-induced *myo*-inositol 1-phosphate accumulation is largely dependent on receptor-mediated turnover of inositol phospholipids.

Specificity of Li^+-induced inositol phosphate accumulation

Sherman *et al.* (1981) reported that Mn^{2+}, as well as Li^+, inhibited *myo*-inositol 1-phosphatase *in vitro*. We assessed the ability of several ions, both bivalent and univalent, to act synergistically with carbachol to stimulate inositol phosphate labelling in cerebral cortical slices. Mn^{2+}, Cs^+ and Rb^+ all produced small increases in inositol phosphate labelling in carbachol-stimulated brain slices. The order of efficacy was $Li^+ \gg Mn^{2+} > Cs^+ = Rb^+$ (5mM for each ion). Sr^{2+} (5mM) markedly inhibited inositol phosphate labelling. It therefore seems that Li^+, when added to the incubation medium, is a particularly effective inhibitor of the hydrolysis of intracellular *myo*-inositol 1-phosphate (results not shown).

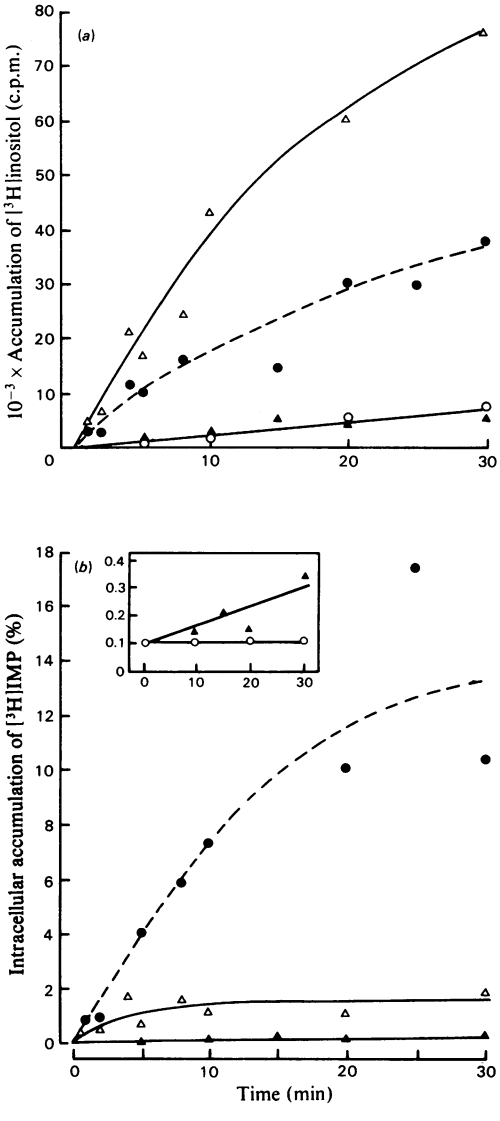

Fig. 5. *Effect of Li^+ on (a) the release of [^{3}H]inositol and (b) the accumulation of [^{3}H]inositol 1-phosphate (IMP) in blowfly salivary glands*

Groups of six salivary glands were incubated with 5 μM-*myo*-[2-^{3}H]inositol for 2h. The glands were washed extensively before being incubated for various times in 200 μl of control medium (○, see inset), 5 mM-Li^+ (▲), 0.5 μM-5-hydroxytryptamine (△) or 5 mM-Li^+ plus 0.5 μM-5-hydroxytryptamine (●). The incubations were stopped by addition of 1 ml of chloroform/methanol (1:2, v/v). Anion-exchange columns were used to separate [^{3}H]-inositol 1-phosphate from [^{3}H]inositol (see Fig. 4). The amount of label present as [^{3}H]inositol 1-phosphate was expressed as a percentage of the total label present in the glands at the end of each incubation. The inset contains an expanded scale to illustrate the control values.

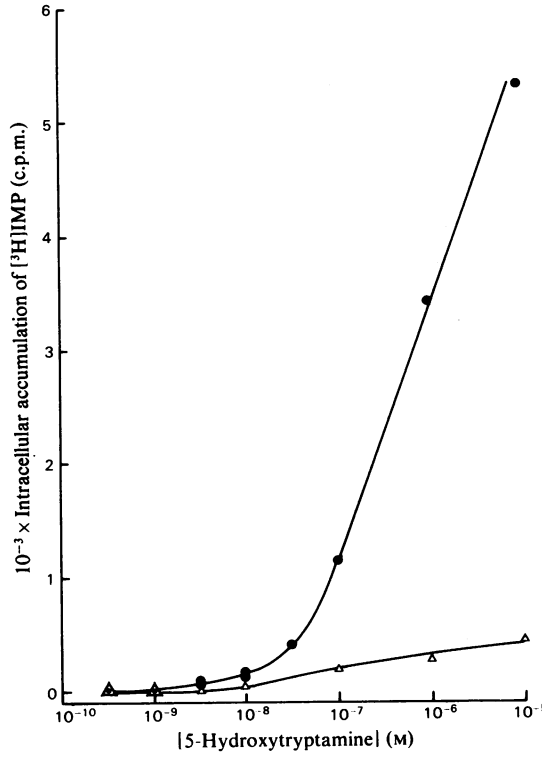

Fig. 6. *Effect of various 5-hydroxytryptamine concentrations on the accumulation of inositol 1-phosphate in the presence or absence of Li^+*

Incubation conditions were identical with those described for the experiment described in the legend to Fig. 5. The prelabelled glands were incubated in solutions containing different concentrations of 5-hydroxytryptamine in either the absence (△) or the presence (●) of 5 mM-Li^+. The extraction and estimation of [^{3}H]*myo*-inositol 1-phosphate (IMP) was similar to that described in the legend to Fig. 5.

Discussion

Li^+ exerts a profound alteration in inositol metabolism by inhibiting the conversion of *myo*-inositol 1-phosphate into *myo*-inositol by the enzyme *myo*-inositol 1-phosphatase (Naccarato *et al.*, 1974; Hallcher & Sherman, 1980). Inhibition of this enzyme will lower the concentration of *myo*-inositol, as has been reported in cerebral cortex during treatment with Li^+ (Allison & Stewart, 1971; Allison *et al.*, 1980). Li^+ also decreases the concentration of inositol in the hypothalamus, hippocampus and caudate nucleus (Allison & Blisner, 1976; Allison *et al.*, 1976, Allison, 1978). Another consequence of inhibiting the phosphatase would be to elevate the intracellular level of *myo*-inositol 1-phosphate and such an effect has also been described in the cerebral cortex (Allison *et al.*, 1976; Allison, 1978; Sherman

et al., 1981) and has been confirmed for the parotid and insect salivary gland in the present study. By inhibiting the phosphatase, therefore, Li^+ not only lowers the concentration of *myo*-inositol but also induces a large accumulation of *myo*-inositol 1-phosphate.

The large amount of *myo*-inositol 1-phosphate that accumulates during the action of Li^+ originates from two main sources (Fig. 7). It can be synthesized *de novo* from glucose, leading to the formation of the L-enantiomer, or it can be released by phospholipase C activity from either phosphatidylinositol or the polyphosphoinositides in the form of the D-enantiomer. The fact that these two sources of inositol lead to the formation of separate enantiomers has enabled Sherman *et al.* (1981) to determine that 90% of the *myo*-inositol 1-phosphate that accumulates in the cerebral cortex during the action of Li^+ is the D-enantiomer. This preponderance of the D- over the L-enantiomer demonstrates that most of the *myo*-inositol 1-phosphate that accumulates with chronic Li^+ treatment comes from the breakdown of the inositol phospholipids (Sherman *et al.*, 1981). In our studies, however, [^{3}H]-inositol-labelled *myo*-inositol 1-phosphate can only arise after phospholipase C attack on inositol phospholipids.

This association between Li^+ inhibition and phospholipid metabolism is intriguing because the phosphoinositides play an important role in those receptors that act through Ca^{2+} as a second messenger (Michell, 1975, 1979; Berridge, 1980, 1981; Michell & Kirk, 1981; Putney, 1981). Such receptor mechanisms may also be responsible for generating other important intracellular signals such as cyclic GMP and metabolites of arachidonic acid such as the prostaglandins, thromboxanes and the leukotrienes (Berridge, 1981). Since there is some uncertainty at present whether the primary action of agonists is to stimulate the hydrolysis of phosphatidylinositol or the polyphosphoinositides (Michell *et al.*, 1981), both possibilities have been included in Fig. 7. For the present argument, it makes little difference which phospholipid is degraded because all the pathways lead to the formation of D-*myo*-inositol 1-phosphate (Fig. 7). Our

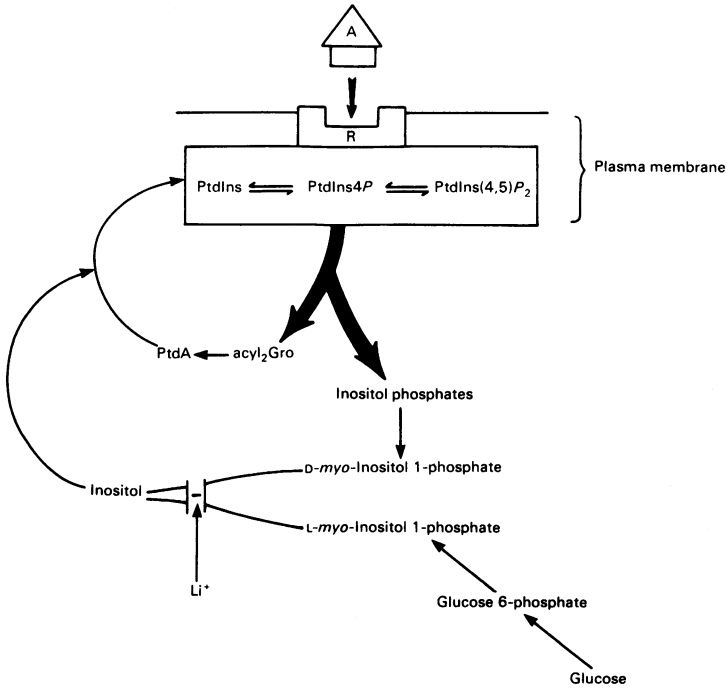

Fig. 7. *A summary of inositol metabolism illustrating the site of Li^+ inhibition on myo-inositol 1-phosphatase*
Agonists (A), such as acetylcholine, 5-hydroxytryptamine, histamine, phenylephrine or Substance P, interact with specific receptors (R) to stimulate the hydrolysis of either phosphatidylinositol (PtdIns) or the polyphosphoinositides phosphatidylinositol 4-phosphate (PtdIns4*P*) or phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to produce diacylglycerol (acyl$_2$Gro) and the corresponding inositol phosphates, which end up as D-*myo*-inositol 1-phosphate. Endogenous synthesis of inositol from glucose yields L-*myo*-inositol 1-phosphate. Both the D- and the L-enantiomers are hydrolysed to inositol by the same enzyme *myo*-inositol 1-phosphatase. This enzyme is strongly inhibited by Li^+, which will act to reduce the supply of inositol that is needed to recombine with phosphatidic acid (PtdA) to reform phosphatidylinositol.

results clearly show that *myo*-inositol 1-phosphate accumulates only slowly in unstimulated cells, but its production accelerates dramatically on activation of receptor mechanisms responsible for hydrolysing inositol phospholipids. The ability of Li^+ to raise the concentration of this phosphate in cerebral cortex *in vivo* could be explained by the endogenous activity of muscarinic receptors, since the effect is abolished by atropine (Allison *et al.*, 1976; Allison, 1978). Similarly, endogenous stimulation of phosphatidylinositol turnover in cerebral cortex is inhibited by atropine (Friedel & Schanberg, 1972).

Only a small proportion of the blowfly salivary gland 5-hydroxytryptamine receptors need to be activated to evoke a maximal secretory response (Fain & Berridge, 1979). Thus, 5-hydroxytryptamine stimulates secretion in the concentration range 1–10nM, which produces a relatively small phosphatidylinositol response (Fain & Berridge, 1979). The 5-hydroxytryptamine-dependent hydrolysis of phosphatidylinositol more accurately reflects receptor occupation since it continues to be stimulated at agonist concentrations far in excess of 10nM. As expected, 5-hydroxytryptamine-stimulated *myo*-inositol 1-phosphate accumulation in the presence of Li^+ requires relatively large concentrations of the agonist (Fig. 6). It therefore seems that accumulation of *myo*-inositol 1-phosphate in the presence of Li^+ is proportional to the agonist-dependent turnover of phosphatidylinositol. This observation may be of fundamental importance not only for providing an explanation for the therapeutic action of Li^+ in controlling manic-depressive illness, but also for introducing a novel and sensitive technique for monitoring the activity of calcium-mobilising receptors. In effect Li^+ can be used to potentiate agonist action in much the same way as the methylxanthines are used to augment the action of those receptors that act through cyclic AMP. The analogy must not be extended too far because, although cyclic AMP clearly functions as a second messenger, there is no evidence so far that *myo*-inositol 1-phosphate performs any role as an intracellular signal.

Despite the fact that Li^+ is used extensively in the control of manic-depressive illness, little is known about its therapeutic mode of action. There is growing evidence that the behavioural symptoms associated with manic-depressive illness may result from changes in the receptor mechanisms operating in various dopaminergic and serotoninergic pathways (Aprison *et al.*, 1978; Treiser & Kellar, 1979; Nagayama *et al.*, 1981; Staunton *et al.*, 1982). If the receptor mechanisms operating in these pathways employ the phosphoinositides, Li^+ might exert its therapeutic action by interfering with the metabolism of these phospholipids (Sherman *et al.*, 1981). The observations reported in this paper support this idea and provide further insights into the possible mode of action of Li^+ within the central nervous system. Perhaps the most important point to emerge from this study is that the ability of Li^+ to distort inositol metabolism is related to receptor-mediated turnover of inositol phospholipid head groups. In a quiescent cell, or when the cell is stimulated at normal agonist concentrations where turnover is low, there is very little accumulation of *myo*-inositol 1-phosphate, but this accumulates rapidly with increasing receptor occupancy (Fig. 6). This implies that the action of Li^+ *in vivo* may be rather selective in that it will be maximally effective against those cells whose inositol phospholipid-linked receptors are being abnormally stimulated. Thus, Li^+ could preferentially affect those receptor pathways that are abnormally active and this may account for its equal effectiveness in controlling both mania and depression.

The next problem to consider is whether Li^+ can bring about an alteration of receptor activity through its inhibitory effect on the *myo*-inositol 1-phosphatase. Inhibition of this enzyme does not cause any immediate effect on receptor action because the accumulation of inositol phosphate was found to increase linearly in the cerebral cortex for at least 60min (Fig. 1). Similarly, Li^+ had no effect on the ability of noradrenaline to stimulate smooth-muscle contraction (Beaty *et al.*, 1981). The fact that Li^+ does not exert an immediate inhibitory effect on these receptor mechanisms is entirely consistent with the clinical observation that several days of chronic treatment are required for its effect (Bunney & Murphy, 1976). The question to consider, therefore, is why such chronic stimulation should lead to a change in receptor activity. As described above, Li^+ inhibits the conversion of *myo*-inositol 1-phosphate into inositol, which is the precursor for the synthesis of phosphatidylinositol (Fig. 7). Our results indicate that Li^+ will be most effective at lowering *myo*-inositol concentration in those cells where receptor-linked inositol phospholipid breakdown is particularly active. It is in these same cells where the demand for *myo*-inositol for resynthesis of phosphatidylinositol to maintain receptor function will be greatest. Continued stimulation of such cells in the presence of Li^+ may gradually deplete the membrane content of phosphatidylinositol and this in turn could decrease the sensitivity of those receptor mechanisms in which inositol phospholipids are important components of the transducing mechanism. Preliminary experiments do indeed suggest that prolonged treatment of parotid glands with carbachol in the presence of Li^+ leads to substantial depletion of the phosphatidylinositol pool. Such a reduction in phospholipid leading to a decline in receptor sensitivity may account for the therapeutic action of Li^+, especially since there are suggestions

that one cause of depression may be an increase in the sensitivity of 5-hydroxytryptamine receptors (Aprison *et al.*, 1978).

A natural extension of this hypothesis is that manic-depressive illness is caused by an alteration in Ca^{2+}-mobilizing receptors rather than in those operating through cyclic AMP. Staunton *et al.* (1982) have suggested that Li^+ may also act on presynaptic dopamine autoreceptors for which there is no identified second messenger. The hypothesis developed above would suggest that these autoreceptors may act through the hydrolysis of phosphatidylinositol.

Many receptors in the periphery (e.g. muscarinic receptors on smooth muscle, pancreas and salivary glands) also hydrolyse phosphatidylinositol as part of their receptor mechanisms and should also be affected by Li^+. However, these peripheral tissues seem to be relatively immune to the inhibitory effect of Li^+. One explanation for this immunity might depend on the fact that these peripheral tissues are protected from Li^+-induced reduction in inositol by obtaining this precursor from the plasma. Indeed, during treatment with Li^+ the plasma level of inositol rises (Allison & Stewart, 1971; Allison, 1978), which would help to protect peripheral tissues against the action of the drug. Since inositol cannot cross the blood/brain barrier (Margolis *et al.*, 1971; Barkai, 1979) the brain is not so protected and must produce its own inositol using the Li^+-sensitive pathways shown in Fig. 7. Hence the brain may be more susceptible to the action of Li^+. The periphery does not escape completely from the action of Li^+, which can induce certain disorders such as polydipsia and hypercalcaemia. The latter is of particular interest, because Shen & Sherrard (1982) consider that the Li^+-induced hypercalcaemia results from a resetting of the 'calciostat' such that it takes a higher level of serum Ca^{2+} to inhibit parathyrin secretion. We consider that Li^+ may act in a similar manner within the central nervous system. Since it interferes with the turnover of inositol and hence the synthesis of phosphatidylinositol, Li^+ may act to reset the sensitivity of those multifunctional receptors that generate second messengers such as Ca^{2+}, cyclic GMP and the prostaglandins.

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